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The Spinal Cord Neural Stem Cell Niche

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1. Introduction

The spinal cord is the caudal portion of the central nervous system (CNS) that extends from the lower part of the brain stem (the medulla) to the cauda equina. It receives several types of sensory information from the joints, muscles, organs and skin and contains the motoneurons responsible for voluntary/reflex movements and for the function of the autonomic nervous system. The spinal cord is divided into i) gray matter, which notably contains motoneurons and interneurons that form the spinal cord circuitry; ii) white matter, which surrounds the gray matter and is made up of ascending and descending longitudinal tracts; and iii) the central canal or ependymal region, which is organized as an oval or round-shaped epithelium whose apical pole abuts the cerebral spinal fluid. The spinal cord is not simply a relay that carries information between the brain and body, but it also contains a complex circuitry that is implicated in the generation and coordination of reflexive responses to sensory inputs. Furthermore, the spinal cord is involved in the formation of rhythmic movements, such as locomotion and swimming in animals. One emerging field of research concerns spinal cord plasticity, as this structure should not be considered a static and hard-wired system. Instead, the spinal cord displays considerable activity-dependent adaptation and, similar to other CNS regions, can learn and remember throughout life (Guertin 2008; Wolpaw 2010; Wolpaw and Tennissen 2001). Plasticity plays an important role in the acquisition and maintenance of motor skills. In pathology, it could be manipulated to alleviate spinal cord lesions that originate from traumas or degenerative diseases.

In parallel with spinal cord plasticity, one field of research that is rapidly growing concerns the presence of neural stem cells and progenitor cells in the adult spinal cord. In this review, I will describe recent findings regarding stem cells and attempt to formulate hypotheses concerning their role in spinal cord physiology and plasticity. The presence of stem cells in the spinal of lower vertebrates, such as salamanders and newts, has been reported for decades. These stem cells are at the basis of the phenomenal regeneration capacity of these animals that is observed when the spinal cord is transected. There are excellent reviews on this topic (for instance, see (Tanaka 2003)), and I will thus focus on the adult spinal cord stem cells in mammals.

2. Discovery and properties of mouse spinal cord neural stem cells

2.1 Spinal cord neurospheres

Definite proof of the presence of neural stem cells in the adult CNS using in vitro assays dates back to the early nineties (Gage, Ray, and Fisher 1995; Reynolds, W., and Weiss 1992). Since then, much attention has been given to stem and progenitor cells in the brain, whereas little is known about these cells in the spinal cord. The persistence of stem cells in this caudal region of the CNS was reported using adherent and non-adherent culture conditions in the late nineties (Shihabuddin, Ray, and Gage 1997; Weiss et al. 1996). The neurosphere assay (Deleyrolle and Reynolds 2009) was instrumental in their discovery, as this assay is particularly suited to demonstrate, at the clonal level, the cardinal properties of stem cells, i.e., multipotentiality, self-renewal and extended proliferation capabilities. Indeed, in 1996 Weiss et al. reported that in mice, 0.1 and 0.6% of isolated thoracic and lumbar spinal cord cells, respectively, that were grown in the presence of FGF2 and EGF, were able to form multipotent and passageable neurospheres (Weiss et al. 1996). Using microdissection and cytometric analysis, these cells were located primarily in the central canal region (Martens, Seaberg, and van der Kooy 2002; Meletis et al. 2008; Sabourin et al. 2009). Progenitor cells with a more limited proliferation potential are also present in the parenchyma (Horner et al. 2000; Kulbatski et al. 2007; Sabourin et al. 2009; Martens, Seaberg, and van der Kooy 2002; Yamamoto et al. 2001). More recently, we were able to show that the dorsal part of the central canal region is enriched in neurosphere-forming cells (Sabourin et al. 2009). Even when clonally-expanded, these neurospheres appear to be heterogeneous entities that are composed of different types of Nestin$^+$ cells, which express various levels of stem cell (CD133), astrocytic (GFAP, Adhl1l1), radial glial cell (CD15, Blbp, Glast, RC2) and oligodendrocytic-lineage (NG2, A2B5, PDGFRα) markers (Fig. 1).

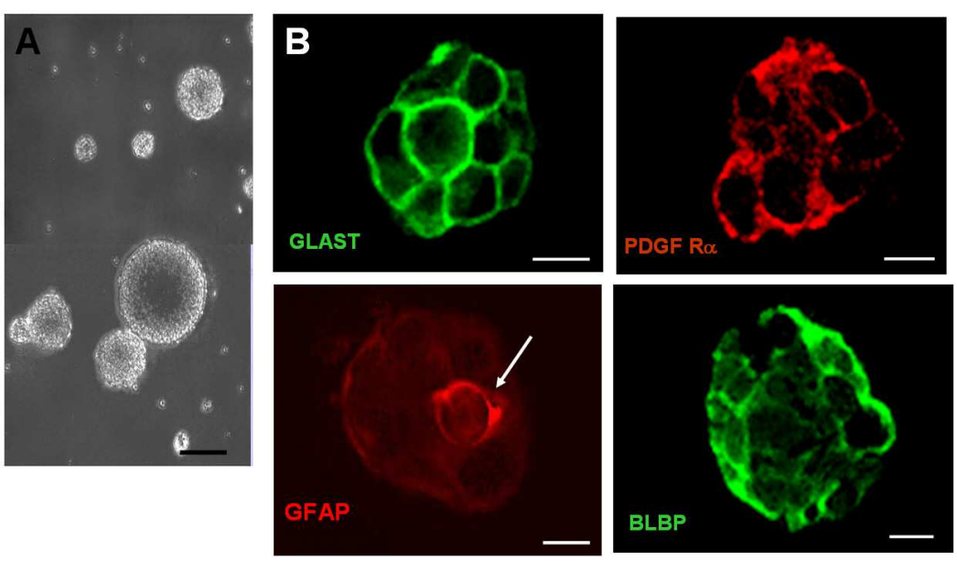

Fig. 1. A) Examples of adult spinal cord-derived neurospheres that were clonally expanded. Note the differences in the sizes of the neurospheres. Scale bar=500 μm. B) Examples of markers that were detected by immunofluorescence in the neurospheres. The white arrow shows a unique GFAP+ cell in a neurosphere. Scale bars=10 μm.

The widespread expression of the latter coincides with the higher propensity of the neurospheres to differentiate into oligodendrocytes vs. neurons in vitro and in vivo after spinal cord injury (SCI) (Kulbatski et al. 2007; Meletis et al. 2008). A few cells that express so-called neuronal markers, such as Map2 and Dcx, are also observed in some neurospheres (Fig. 2).

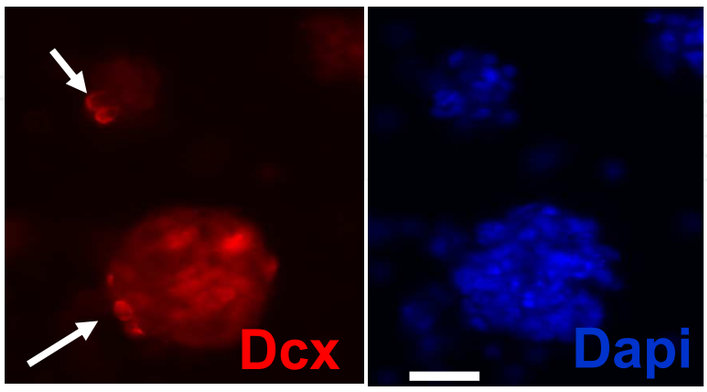

Fig. 2. Example of Dcx+ cells detected in spinal cord-derived neurospheres. Scale bar= 100 μm.

Often, several of these markers are coexpressed in the same cells, which may reflect the presence of intermediates and uncommitted states of differentiation. GFAP$^+$ cells in these spheres (Fig. 1) should not be considered to be astrocytic-differentiated cells but rather, as they frequently co-express immature markers (CD133, CD15), these cells likely represent GFAP$^+$ neural stem cells. Within neurospheres, only a fraction of the cells, typically between 1 to 10%, are able to generate new neurospheres and are considered to be bona fide neural stem cells. Other cells are considered to be progenitors because they give rise to small neurospheres with limited proliferative and self-renewal capabilities (Louis et al. 2008). The prospective isolation of stem vs. progenitor cells in neurospheres remains challenging, as no definitive cell surface marker has been clearly identified to distinguish these two types of cells.

Notably, as observed for neurospheres that were derived from different brain regions (Armando et al. 2007; Conti and Cattaneo 2010), neurospheres derived from different parts of the spinal cord have different growth and differentiation properties (Kulbatski and Tator 2009; Sabourin et al. 2009). We showed that neurospheres that were derived from the cervical, thoracic and lumbar regions maintained their expression of specific and different rostro-caudal combinations of developmental homeogenes of the Hox family. These data indicate that even after several passages in vitro, these cells maintain molecular cues from their original position. This phenomenon might affect considerations regarding cellular therapy with adult neural stem cells because not all cells might have equivalent capacities to replenish cell loss and to integrate into the adult host tissue. In addition to Hox genes, the adult spinal cord neurospheres express high levels of a set of transcription factors, including Dlx2, Nkx2.2, Nkx6.1, Olig2, Pax6, Sox2, Sox4 and Sox9 (Moreno-Manzano et al. 2009; Sabourin et al. 2009; Yamamoto et al. 2001) which are involved in spinal cord embryonic development. This expression likely reflects the maintenance, in adult stem cells, of embryonic transcriptional programs and active signaling pathways. Yet, these neurospheres appear to express only a limited range of developmental gene networks, as the transcription factors that are involved in motoneuron development, such as Islet1, Lim1 and HB9, are not

expressed (Yamamoto et al. 2001), which illustrates a somewhat restricted fate for these cells. Indeed, upon differentiation in vitro, these cells primarily generate GABAergic neurons, oligodendrocytes and astrocytes (Moreno-Manzano et al. 2009; Sabourin et al. 2009). Importantly, these cells appear to remain competent to respond to morphogens to redirect their differentiation into other neuronal cell subtypes. Indeed, treatment with embryonic morphogens that are involved in spinal cord caudal regionalization and motoneuron development, i.e., retinoic acid and sonic hedgehog, was able to drive their differentiation toward electrophysiological active motoneurons (Moreno-Manzano et al. 2009).

2.2 Neurosphere differentiation

The differentiation of adult spinal cord neurospheres into neuronal and glial cells is generally achieved by plating them onto an adhesive substrate and by removing growth factors. This differentiation occurs even without the addition of serum, suggesting that endogenous cytokines are implicated in the differentiation process. Indeed, we found that undifferentiated and differentiated neurospheres expressed at a high level, a wide range of endogenous cytokines (Deleyrolle et al. 2005). The expression of several of them is controlled by the FGF2 and EGF which are present in the medium. Notably, upon differentiation, a striking upregulation of astrocytic differentiating factors, such as BMP4, BMP6 and CNTF, are observed. The active role of these endogenous cytokines is illustrated by inhibition experiments that impair neurosphere differentiation (Deleyrolle et al. 2005). This production of endogenous cytokines by adult spinal cord stem cells could be considered to be beneficial in the context of cellular therapy because these cytokines have been shown to actively participate in the therapeutic effects observed following neural precursor cell transplantation (Pluchino et al. 2003; Redmond et al. 2007). In contrast, these endogenous factors, together with host factors, might also contribute to the absence or the low rate of neuronal formation, which is frequently observed in grafting experiments. Further investigations of the neurosphere differentiation process would yield important insights on how to direct the fate of endogenous and exogenous neural stem cells into the most appropriate cell type to achieve rational and effective spinal cord cellular therapy.

3. The central canal niche and identity of adult spinal cord stem cells

Many adult organs harbor a pool of stem cells in specialized structures called niches. These act as a nest and a barrier to protect, nourish and regulate the fate of stem cells. They do so by providing, in highly organized structures, cellular and molecular cues suitable for the strict control of stem cell properties (e.g., self-renewal, differentiation, quiescence). Typically, these niches contain a high level of canonical developmental signaling pathways, notably, BMP, SHH, Wnt and Notch (Li and Clevers 2010). These signaling pathways precisely regulate the proliferation/quiescence, differentiation/self-renewal and migratory/stationary balances of the stem cell pool. In addition, their particular architecture favors interactions between stem cells and specific cells, such as vascular cells. Historically, the best-characterized niches in mammals are the hematopoietic and intestinal niches, and more recently, the CNS niches have been studied. In the brain, the hippocampus, the recently discovered sub-callosal zone and the subcortical white matter contain neural progenitors, whereas bona fide stem cells that are capable of sustained proliferation are preferentially found in the subventricular zone (SVZ) (Seaberg, Smukler, and van der Kooy

2005). In the latter, contacts with the cerebral spinal fluid (CSF) located in the ventricles appear to be essential for the maintenance of SVZ stem cells (Lehtinen et al. 2010). Stem and progenitor cells have also been identified in the peripheral nervous system, i.e., in the carotid body, the enteric nervous system and the adult dorsal root ganglia (Pardal et al. 2007; Schafer, Van Ginneken, and Copray 2009; Singh et al. 2009). Whereas there have been a tremendous number of publications on the brain niches, few have addressed this issue in the spinal cord.

3.1 Cellular diversity in the central canal region

The central canal region is composed of several cell types, which are located either in direct contact with the lumen or in a subependymal position, evoking a pseudo-stratified epithelium (Fig. 3). However, a distinct subependymal layer, as observed in the SVZ, is not present. Ependymocytes are the primary cell type found around the central canal. A second frequently observed cell type is the tanycyte (also referred to as radial ependymocytes) (Seitz, Lohler, and Schwendemann 1981), which is mostly observed on the lateral sides of the central canal region. This ependymal cell type sends a long basal process that terminates at the blood vessels (Horstmann 1954). Tanycytes are in contact with the lumen, but their soma can either be subependymally or ependymally located (Meletis et al. 2008; Rafols and Goshgarian 1985). As in the brain, they bridge the CSF to the capillaries, thereby providing a potential link between the CSF and the blood.

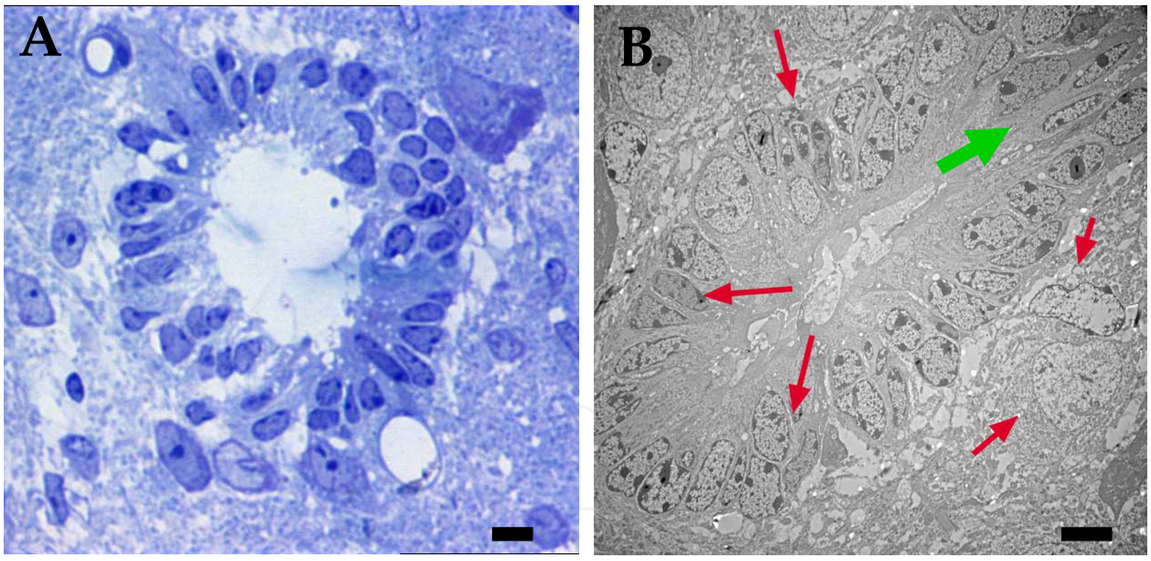

Fig. 3. A) Semi-thin section in the adult mouse thoracic spinal cord ependymal region (toluidine blue staining). Note the diversity of the cell types in contact with the lumen or in a subependymal position. B) An electron micrograph showing different types of cells around the central canal (red arrows). The green arrow indicates the dorsal region of the canal, which contains densely packed cells.

The central canal region contains also neuronal-like cells which contact the CSF. These are very common and well-described in several lower vertebrates especially fishes and amphibians (see for review (Vigh et al. 2004)). In mammals, their presence has been reported

in several species including primates and rodents (Hugnot and Franzen 2010). These cells are sporadically distributed around the canal with a soma in an ependymal or subependymal position and they send a single thick dendritic-like process terminated by a large bulge in the lumen (Sabourin et al. 2009). Even in adults, these cells continue to express PSA-NCAM (Marichal et al. 2009; Seki and Arai 1993), Dcx (Marichal et al. 2009; Sabourin et al. 2009) and GAP43 (Stoeckel et al. 2003), three proteins that are involved in plasticity and migration, suggesting that they are endowed with some degree of immaturity. In rodents, these neuronal cells appear to be mainly GABAergic, and their function remains elusive. The expression of acidic pH-activated channels suggests that these cells might be implicated in CSF homeostasis (Huang et al. 2006; Marichal et al. 2009). They could also act as mechanoreceptors, which are sensitive to CSF pressure or flow or to spinal cord flexion. Importantly, these cells are not produced from continuous adult spinal cord neurogenesis, and a study performed in rats demonstrated that they are in fact produced during embryogenesis (Marichal et al. 2009).

The dorsal and ventral regions of the central canal display a divergent organization of a higher density of cells with a radial morphology, which are situated in ependymal and subependymal positions (Hamilton et al. 2009; Meletis et al. 2008; Sabourin et al. 2009). GFAP+ cells are frequently observed in these regions. Particularly in the dorsal region, some of these GFAP+ cells have long basal processes that extend along the dorsal midline up to the dorsal column white matter or pial surface (Bodega et al. 1994; Hamilton et al. 2009; Sabourin et al. 2009). GFAP+ cells can also be observed in the lateral region of the canal. These GFAP+ cells can lie in the ependymal layer adjacent to the canal lumen but are often located in a subependymal position, where they send a process toward the canal. Transgenic mice expressing a hGFAP promoter-GFP construct are particularly suited to visualize these cells (Fig. 4). Interestingly, some cells that express radial glia markers, such as CD15 or BLBP (some of which are GFAP+), are also occasionally detected in the dorsal region (Fig. 5).

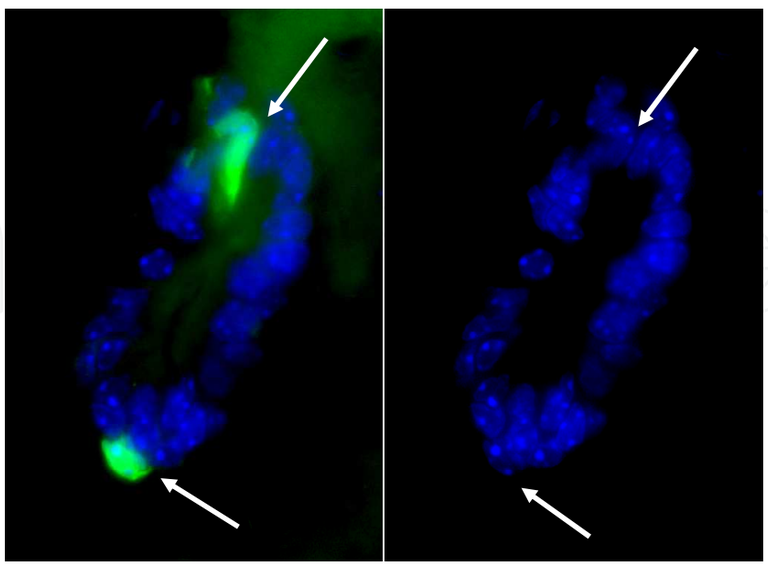

Fig. 4. Examples of GFP+ cells detected in the dorsal and ventral regions of the central canal region of hGFAP-GFP mice (Nolte et al. 2001). The dorsal GFP+ cell is located in the ependymal layer, whereas the ventral cell is located in the subependymal layer.

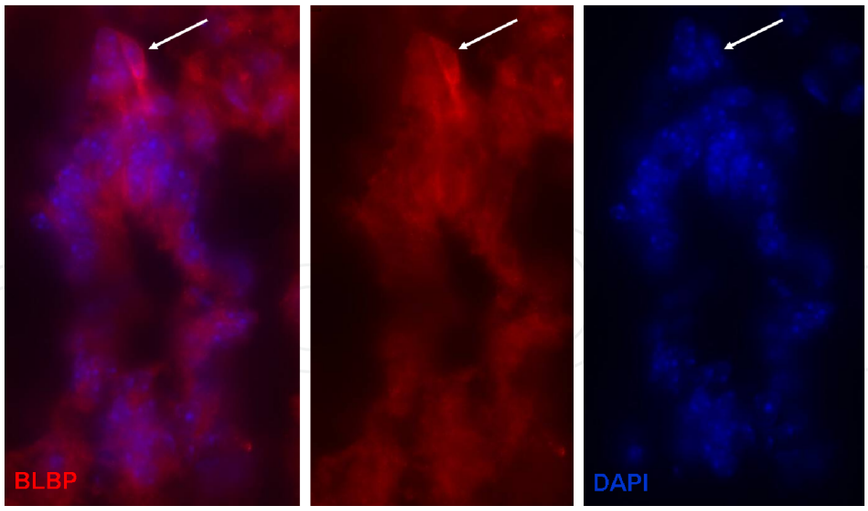

Fig. 5. Example of a subependymal BLBP+ cell detected in the dorsal part of the central canal of the mouse lumbar spinal cord (arrow).

The central canal region is surrounded by an abundant vasculature, and cellular proliferation within the niche occurs in close proximity to the vessels (Hamilton et al. 2009). This observation is consistent with the recently well-described interactions between neural stem/progenitor cells and endothelial cells (the so-called neurovascular niche (Palmer, Willhoite, and Gage 2000)).

Collectively, it appears that the central canal region is composed of several cell types that are localized at specific locations and express characteristic markers with different morphologies and potentially different functions. A schematic drawing of the lumbar central canal region is presented in Fig. 6.

3.2 Signaling within the niche

The persistence of stem cells within the central canal region implies that specific pathways are active or readily activated in the niche. These pathways will maintain the proliferation potential and multipotency of stem cells. Equally, the niche may contain molecules that act in a passive mode to protect stem cells from local or circulating growth and differentiation factors. Our lab used two approaches to identify important cues in the mouse spinal cord niche. First, we used immunofluorescence to screen for the presence of receptors, ligands and transcription factors that are associated with the Notch, SHH, Wnt and epithelial-mesenchymal transition (EMT) pathways. Second, we extensively screened online gene expression databases (notably, Allen Brain and the Gensat Atlas) for genes that are specifically expressed in the spinal cord central canal region. These approaches allowed us to identify the expression, at the transcript and/or the protein level, of several molecules involved in the Notch (Jagged, Hes1), Wnt (Wnt7a, Fzd3), BMP (DAN, BMP6) and Hedgehog (SHH) pathways (Hugnot and Franzen 2010; Sabourin et al. 2009). These genes are expressed by most of the cells in the central canal region or by restricted subpopulations. Unexpectedly, we also found that cells in this region highly expressed Zeb1 (Sabourin et al. 2009) (also known as δ-EF1, TCF8, AREB6), a zinc finger-homeodomain transcription factor, which has been described as an important regulator of EMT (Liu et al. 2008). Zeb1 protein is

detected in the majority of cells surrounding the lumen but is present at higher levels in the cells that are located in the medial dorsal region, notably, the previously mentioned GFAP+ cells. Zeb1 is also detected in subpopulations of cells in the white and grey matter. Zeb1 is involved in the regulation of several cellular processes, such as migration, senescence and apoptosis. It exerts control by acting as a repressor for a number of genes, such as P15Ink4b, P21 Cdkn1, E-cadherin, CRB3 and myogenic transcription factors (Browne, Sayan, and Tulchinsky 2010). Conversely, it also acts as an activator for a group of genes that are typically expressed in mesenchymal cells, such as collagens, vimentin and smooth muscle actin (acta2) (Nishimura et al. 2006). As the two latter proteins are expressed in the central canal and SVZ cells (Sabourin et al, 2009), their expression might be under the control of Zeb1. Consistent with a role in adult precursor cells, Zeb1 and 2 are expressed by neurosphere cells derived from the adult spinal cord. These transcription factors are required for neurosphere formation and expansion because we demonstrated that the transfection of a dominant-negative form of Zeb1 and 2 induced massive apoptosis in vitro. In Drosophila, the Zeb1 orthologous protein Zfh-1 was recently shown to have a critical role in the maintenance of the somatic stem cell compartment in the testis stem cell niche (Leatherman and Dinardo 2008). These data suggest that the role of this family of transcription factors in the maintenance of immature properties has been conserved throughout evolution.

3.3 Identity of stem cells in the niche

Considering the diversity of cell types in the central canal region, the precise identity of the cells that are able to generate passageable neurospheres needed to be addressed by methods based on cell purification. A common and powerful technique is based on the cytometry of cells isolated from GFP transgenic animals, where a specific cell type is tagged using a cell-specific promoter. Alternatively, specific membranous markers and antibodies can be exploited for purification; however, this method can be challenging for studies of the adult spinal cord, as enzymes required for cellular dissociation could damage membrane-bound markers and lead to erroneous conclusions. To explore whether the GFAP+ cells we observed in the central canal region were endowed with stem cell properties, we used the hGFAP-GFP transgenic line established by Dr Kettenmann's group (Nolte et al. 2001). Sorting GFP+ cells by cytometry revealed that compared with GFP^-, the vast majority of neurospheres (>80%) are derived from GFP+ cells (Sabourin et al. 2009). In total, 0.2% of GFP+ cells were able to generate neurospheres. This frequency might appear low, but one must consider that the purification of GFP+ cells from these animals cannot discriminate GFP^+ cells located in the central canal region from those of the parenchyma, which are much more numerous. Consistent with our hypothesis that central canal GFAP+ cells are endowed with stem cells properties, we found that most primary neurospheres derived from these transgenic animals contained one or several GFAP+/GFP+ cells. This result supports the notion that as observed in the SVZ, the central canal cells with astrocytic features have neural stem cell properties. Another team conducted a second transgenic mouse approach with FoxJ1-GFP animals (Meletis et al. 2008). FoxJ1 expression is restricted to the central canal region, and it was assumed that there was no expression in GFAP+ cells. Using this line, Meletis et al. reported that the majority of spheres are derived from the GFP^+ fraction with an approximate frequency of 0.2% of GFP+ cells giving rise to neurospheres. As no GFAP+ cell was observed around the canal in this study, it was concluded that adult spinal cord neural stem cells are

GFAP- ependymal cells. Yet, in contrast with the GFP+ population purified from hGFAP-GFP animals that contain GFP+ cells from both the central canal region and the parenchyma, the cells obtained from the FoxJ1-GFP mice appear to be exclusively derived from the cells in the central canal region. Thus, the obtained frequency of 0.2% for neurosphere formation in FoxJ1+ cells signifies that only a small subpopulation of undefined ependymal cells (1/5000) would be endowed with neural stem cell properties. Moreover, a recent elegant transcriptome analysis from Beckervordersandforth, et al. clearly indicated that in the SVZ, GFAP+ neural stem cells highly expressed FoxJ1 (Beckervordersandforth et al. 2010).

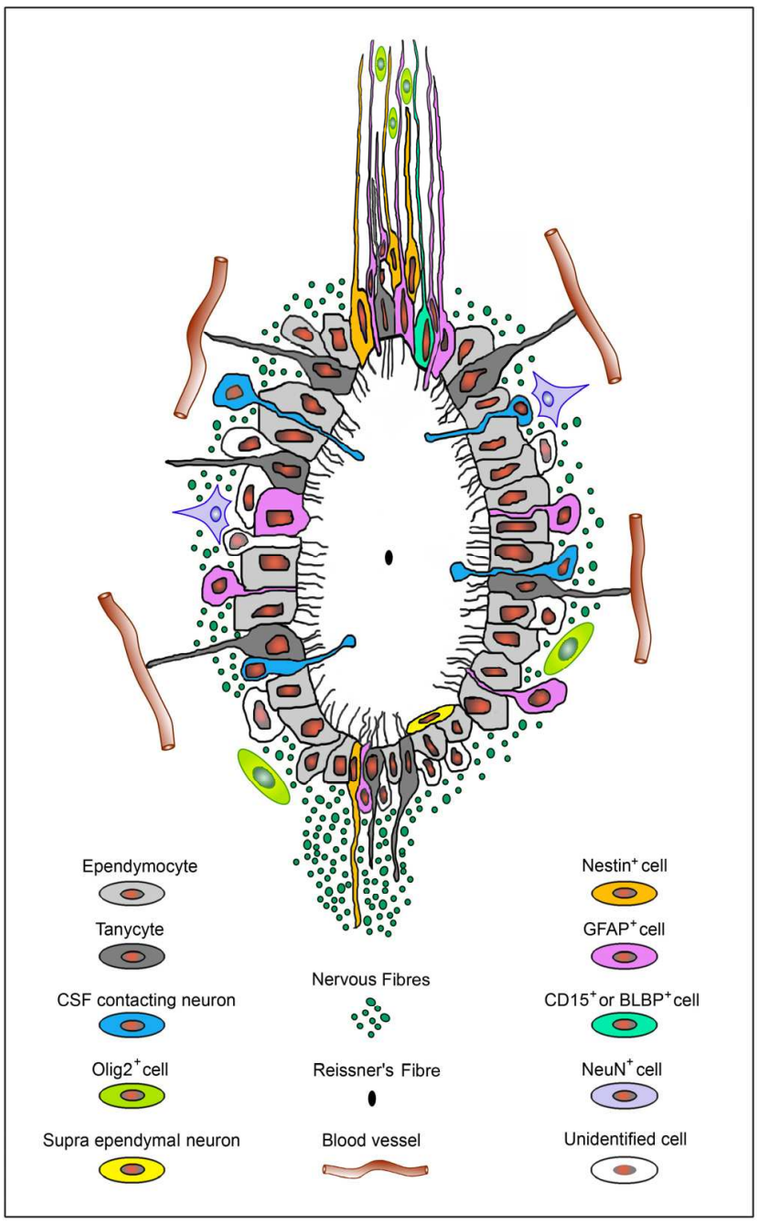

Fig. 6. Schematic drawing of the adult mouse ependymal region. Reproduced from (Sabourin et al. 2009)

Using immunofluorescence for FoxJ1 on hGFAP-GFP sections, we could readily observe double-labeled cells (Fig. 7, unpublished data). This indicated that in addition to the ependymocytes, the FoxJ1 transcription factor is also present in neural stem cells as also suggested by the study reported by Jacquet, BV et al (Jacquet et al. 2009).

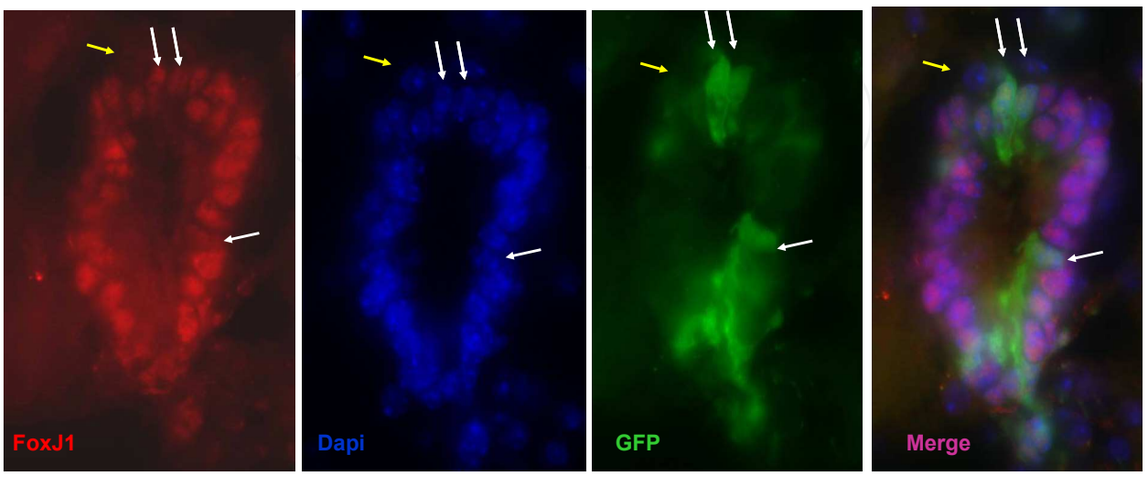

Fig. 7. Immunodetection of FoxJ1 in the cells around the central canal of the hGFAP-GFP adult mouse. The white arrows point to two GFP+ cells expressing FoxJ1. The yellow arrow indicates a subependymal FoxJ1-negative cell.

In the SVZ, neural stem cells are currently primarily considered to be CD133+ GFAP+ cells, whereas ependymocytes are endowed with a more restricted proliferative potential. In the spinal cord, most of the cells around the canal are $CD133^+$ (Sabourin et al. 2009), and in primary neurospheres, CD133+ GFAP+ cells are frequently observed. Thus, it is likely that GFAP+ CD133+ cells in the adult spinal cord represent all or at least a substantial fraction of neural stem cells. Considering that in the SVZ, $GFAP^-$ transit amplifying cells can be converted into stem cells with EGF (Doetsch et al. 2002), the possibility still exists that a fraction of neurospheres could be derived from central canal $GFAP^-$ cells.

3.4 Developmental origin of the central canal region

Neurospheres derived from the mouse adult lumbar spinal cord express the Nkx6.1 transcription factor (Sabourin et al. 2009). In vivo, most cells around the central lumen also express this factor; however, there was a variable level of expression and the CSF-contacting neurons displayed the highest staining in our studies. During spinal cord development, Nkx6.1 is expressed in the ventral neural tube, which contains progenitor cells of three main neuronal classes: V2, MN and V3 interneurons (Briscoe et al. 2000). Using marker analysis, Fu et al. concluded that the central canal region in mice and chicks is derived from the $Nkx6.1^+$ $Nkx2.2^-$ domain, which expresses the Olig2 transcription factor (Fu et al. 2003). The potential role of Nkx6.1 in the formation of the central canal niche might be to maintain adult stem cells, as suggested by its highly conserved expression in the chick, rodent and human (Fig. 9).

4. Neural precursor cells in the adult human spinal cord

Much of our knowledge of adult neural stem cells is derived from studies performed in rodents, but much less is known about these stem cells in humans. Adult neural stem and progenitor cells have been isolated from different parts of the human brain, i.e., the olfactory bulb, the SVZ, the hippocampus and the cortex, using the neurosphere assay (Arsenijevic et al. 2001; Roy et al. 1999; Roy et al. 2000; Nunes et al. 2003; Kukekov et al. 1999; Pagano et al. 2000; Scolding, Rayner, and Compston 1999; Sanai et al. 2004; Akiyama et al. 2001). There are major differences between the rodent and primate brain, not only concerning size and organization but also for the cell diversity and SVZ organization. Consequently, we set out to explore the organization of the central canal region and the presence of neural stem cells in the adult human spinal cord (Dromard et al. 2008). For this purpose, we used lumbar spinal cords from brain-dead organ-donor patients (24–70 years old) (Fig. 8) and cervical spinal cords from autopsy tissues.

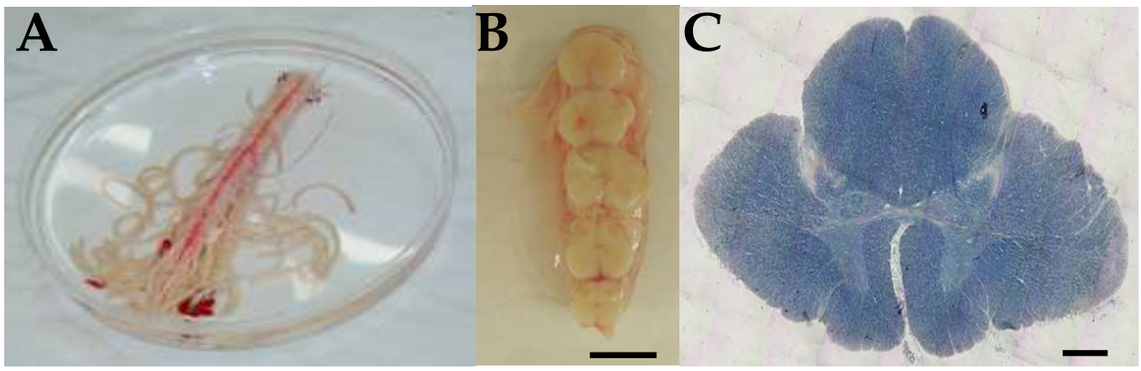

Fig. 8. A) Dissected human spinal cord (T9 thoracic region to caudal end). B) Thoracic, lumbar and sacral spinal cord fragments. Scale bar= 10 mm. C) Luxol staining of a thoraco-lumbar section. Scale bar=1 mm.

Histology and immunohistology studies revealed both similarities and dissimilarities between rodents and man. First, in contrast to mice, the central canal of the human spinal cord is often occluded (Dromard et al. 2008), as previously reported by (Fuller and Burger 1997; Milhorat, Kotzen, and Anzil 1994), and the ependymal region appeared disorganized, with the frequent presence of rosettes or microcanals. Reminiscent of the reported difference between the SVZ in rodents and humans (Quinones-Hinojosa et al. 2006), the human central canal is surrounded by a hypocellular region containing a high density of GFAP filaments and nerve fibers (Dromard et al. 2008). Equally contrasting with rodents, a cluster of Nestin$^+$ subependymal cells was repeatedly observed in the ventral regions of cervical and lumbar spinal cords. Immunolabeling for CD15, Nestin, Nkx6.1, PSA-NCAM and Sox2 revealed, as observed in rodents, that this region retains immature features and is composed of several cell types in contact with the canal or in a subependymal position (Fig. 9).

Whereas most central canal cells expressed Nkx6.1 and Sox2, Nestin, GFAP and CD15 expression was restricted to cell subpopulations (Fig. 9). Nestin is only expressed by a fraction of cells in direct contact with the lumen or in a subependymal position. In contrast, to rodents, GFAP+ cells are numerous and more frequently located in direct contact with the

lumen. Strikingly, CD15 expression is most often observed in cells in the dorsal region of the canal. In one lumbar spinal cord, we observed the expression of PSA-NCAM by a subpopulation of cells primarily localized on the ventral side of the central canal region. Taken together, these results represent the first analysis and reveal the complexity of this region in humans; however, further characterization, notably with new markers for neural stem cells, such as Bmi1, is required.

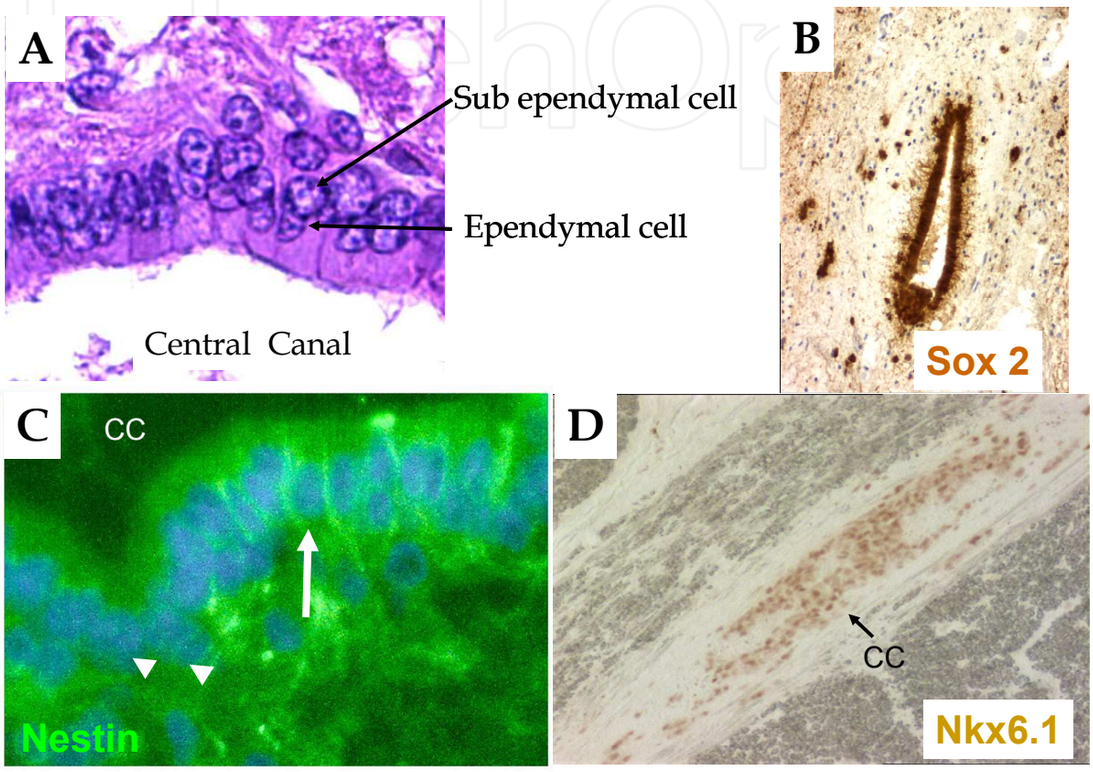

Fig. 9. A) Hematoxylin and eosin staining of a lumbar spinal cord section demonstrating the presence of several layers of cells in the central canal region. B, C, D) Immunodetection of the indicated protein. Note the intense Sox2 staining around the lumen (B), the presence of Nestin+ (arrows) and Nestin- (arrowheads) cells (C) and the expression of Nkx6.1 by central canal cells (D). In D, the central canal region is disorganized without a well-delimited lumen. These data may represent the actual organization or an artifact that occurred as a consequence of tissue processing for histology.

To explore the presence of neural stem or progenitor cells in the human spinal cord, we used the classical neurosphere assay. As compared with rodents, the frequency of neurosphere formation was at least 10 times lower (0.01-0.03 % of isolated cells). These neurospheres were Nestin+ Sox2+ and contained proliferative cells, as evidenced by Ki67 labeling and BrdU incorporation. In one sample, we were able to separate the central region from the surrounding tissue and observed that most of the spheres were derived from the central region. Upon differentiation (Fig. 10), these spheres generated glial (predominantly GFAP+ cells) and neuronal cells. These cells were mostly GABAergic, but some 5-HT neurons could be observed.

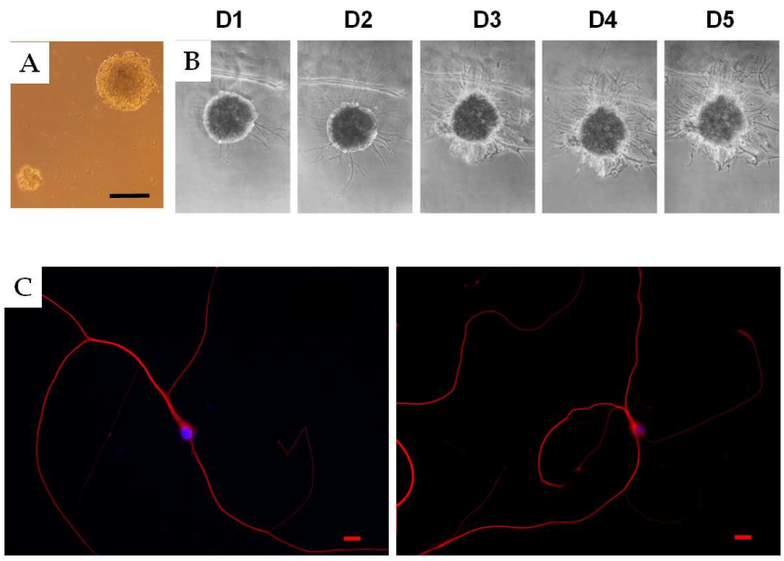

Fig. 10. A) Example of primary neurospheres derived from culturing dissociated human spinal cord cells (two weeks of culture). Scale bar= 500 µm. B) Neurosphere differentiation obtained by plating on an adhesive substrate for 5 days (D1-D5). C) Example of long Map2+ cells observed in differentiated neurospheres. Scale bars= 20 µm.

Importantly, despite intense attempts, we were not able to passage these neurospheres. This suggests that either these cells were progenitors with a limited proliferation potential or that the culture conditions were not adequate to sustain human neural stem cells self-renewal. As previously reported the long-term propagation of human neural stem cell culture is more challenging than that of rodents and might require specific techniques, such the absence of complete dissociation during the passaging process (Svendsen et al. 1998).

In addition to the neurosphere method, adherent conditions and a specific media containing serum could be used to isolate and propagate neural stem cells from the human temporal cortex and the hippocampus (Walton et al. 2006). We recently used these conditions as an attempt to isolate stem cells from the adult human spinal cord (Mamaeva et al. 2011). This attempt resulted in the isolation of a proliferating Nkx6.1+ Nestin+ cell population, which could be maintained for up to 9 passages. However, an in depth cellular characterization showed that these cells were acta2+ caldesmon+ calponin+ smooth muscle cells. These cells also displayed mesenchymal cell features, as evidenced by the high level of expression of the two transcription factors Snai2 and Twist1. We also observed that in vivo, Nkx6.1 was expressed by a subset of spinal cord vascular cells in addition to the central canal cells. Attempts to differentiate these cells into glial cells, neurons, chondrocytes and adipocytes were unproductive; however, these cells could readily become mineralized (formation of CaPO4) when placed under osteogenic conditions. The calcification of CNS muscular cell vessels has been observed since 1884 (Compston 2007; Obersteiner 1884) in pathological situations or as part of the aging process in the brain (Makariou and Patsalides 2009). These cells constitute a useful model for studying CNS vessel calcification in vitro.

Finally, preliminary work from our lab suggests that in addition to the central canal niche, neural precursor cells might also reside in the parenchyma. For instance, Dcx^{+} cells can be

observed in the white matter, whereas scattered Sox2+ are notably present in the gray matter (Fig. 11). These results indicate that immature neural cells might represent a significant population of spinal cord cells with a completely unknown function.

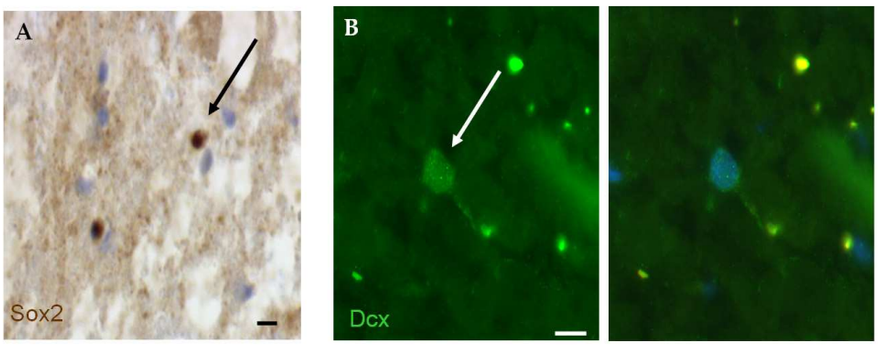

Fig. 11. A) Example of Sox2+ cells detected in the gray matter of the adult human spinal cord. B) Example of one Dcx+ cell detected in the white matter of the adult human spinal cord. Scale bars= 10 µm.

5. Role of the niche in spinal cord physiology and plasticity

5.1 Proliferation in the niche

The spinal cord elongates extensively during the post-natal period, and its size increases 2.5-fold in mice from birth to 13 weeks (Sabourin et al. 2009). In parallel with this extension, active proliferation is detected postnatally in the central canal region, notably in the ependymocytes, and subsequently declines at 12-13 weeks, which corresponds to the end of spinal cord elongation (Fu et al. 2003; Sabourin et al. 2009). This production of new cells is likely to be necessary for extending the length of the central canal. In addition, as observed postnatally in the brain SVZ (Suzuki and Goldman 2003), it is likely that cells are produced from the central region for myelination or for integration into the developing spinal cord circuitries. In contrast to young animals, in adult rodents and humans, proliferation is low or absent in the ependymal region. Thus, it appears that in adults, most central canal cells are quiescent, meaning that they are in an arrested but reversible state. Consistent with the proliferative decline observed in the postnatal period, we also noted that several markers, such as nestin, cadherin-13, and Sox4, are more highly expressed in young animals than in adults (Sabourin et al. 2009).

The central canal cells could, however, readily re-initiate proliferation in spinal cord traumas (Vaquero et al. 1981) and in several models for neurodegenerative diseases, such as in multiple sclerosis (Danilov et al. 2006) and amyotrophic lateral sclerosis (Chi et al. 2006). In these situations, cells migrate from the central canal region toward the lesion sites by a mechanism that may involve the SDF1/CXCR4 pathway which is present in these cells (Hugnot and Franzen 2010; Shechter, Ziv, and Schwartz 2007). Their fate appear to become mostly macroglial cells and not neurons, despite an increased expression of the transcription factor Pax-6 (Yamamoto et al. 2001; Johansson et al. 1999; Coksaygan et al. 2006). Indeed, they preferentially differentiate into GFAP+ astrocytes contributing to scar formation (Meletis et al. 2008; Johansson et al. 1999; Takahashi et al. 2003; Mothe and Tator 2005) and

to some extent into myelinating Olig2 oligodendrocytes (Meletis et al. 2008) making them interesting candidates for myelin degeneration diseases like multiple sclerosis.

Collectively, these data emphasize an important postnatal role for the cells of the central canal region. In adults, these cells enter a dormant state, which could be reactivated in specific physiological situations or under pathological conditions.

5.2 Spinal cord central canal niche, physical exercise and spinal plasticity

Whereas the role of forebrain stem cell niches in memory and learning is being elucidated, the functions of the spinal cord niche, if there are any, remain elusive in adults. One important observation reported by (Cizkova et al. 2009; Foret et al. 2009; Krityakiarana et al. 2010) is that physical exercise (treadmill training and wheel running) can reactivate proliferation within the niche and increase nestin expression. The identity and fate of newly formed cells needs to be fully addressed using reliable labeling techniques, such as permanent genetic lineage tracing with Cre recombinase. One possibility is that the cells in the niche generate new CSF-contacting neurons upon training. Work by Marichal et al. showed that the latter are at different stages of maturation, which raises the possibility that they are in "standby mode" and, under some circumstances (e.g., injury or training), might complete maturation to integrate spinal circuits (Marichal et al. 2009). Another exciting possibility would be that newly formed cells contribute to spinal cord activity-dependent plasticity. For instance, in addition to the hippocampus, long-term potentiation (LTP) has recently been detected in the spinal cord dorsal horn following natural noxious stimulation (Randic, Jiang, and Cerne 1993; Rygh et al. 1999). In the brain, the experimental modification of neurogenesis alters hippocampal LTP (Snyder, Kee, and Wojtowicz 2001; Saxe et al. 2006). Similarly, one could consider the implication of cells generated in the central canal niche for dorsal horn LTP. Along this line, Shechter et al. reported the presence of newly formed GABAergic Dcx+ in the dorsal spinal cord region (Shechter, Ziv, and Schwartz 2007). Further investigations are needed to understand the origin and role of these cells.

The capacity of the spinal cord to learn and memorize is now acknowledged (Guertin 2008; Wolpaw 2010; Wolpaw and Tennissen 2001). For instance, basic spinal cord functions, such as stretch reflexes, can be conditioned even after complete spine transection. Locomotion is another flexible function. It is largely generated by spinal cord circuits localized, notably, in the lumbar region and called central pattern generators (Guertin and Steuer 2009). These circuits generate basic signals for walking. After spinal cord transection, these networks do not die and can be modified and reactivated by several days of specific training sessions. This learning allows rats and cats to display involuntary but structured walking on a treadmill (Rossignol et al. 2009). Whereas these examples of plasticity are largely mediated by synaptic modulations, one could also envision that long-term adaptation would involve the recruitment of new glial and potentially neuronal cells. By harboring cells with a high proliferation and differentiation potential, the central canal niche would be a particularly suited source for providing such new cells.

6. Conclusion

In addition to SVZ and hippocampus, the spinal cord central canal region constitutes a-third adult neural stem cell niche (Hugnot and Franzen 2010). In contrast with the brain, a

sustained cell production is not observed in adults in which the niche appears to be largely dormant. In several spinal cord pathologies, the niche reactivates promptly to generate new astrocytes and oligodendrocytes, which participate in repopulating the damaged tissue in addition to forming the glial scar. It appears, therefore, that cells in the niche are in a stand-by but ready-to-go mode. This outstanding capacity is associated with the presence of fetal features in the niche that is well illustrated by the maintenance of activated developmental pathways and genes. These pathways will maintain cells at a high potential for proliferation, differentiation, delamination and migration. Far from being a simple layer of homogenous cells, both in man and in rodents, this region is highly organized and is composed of several cell types. Further work is required to exactly determine the different properties of these cells, which is particularly true for the Dcx^+ CSF-contacting neuronal cells whose function is completely unknown. New cell-specific GFP transgenic mice together with fate mapping using Cre-Lox techniques will be valuable tools to address these issues.

Regarding spinal cord lesions, one important goal is to accurately control and redirect stem cell fate to endogenously repopulate the lesioned tissue with the appropriate cell type. This control and redirection would be an invaluable step for designing new therapeutic strategies against pathologies, such as amyotrophic lateral sclerosis or multiple sclerosis. It would require a detailed understanding of the molecular mechanisms involved in controlling spinal cord neural stem cell fate together with a broad knowledge of how stem cells generate specific neuronal cell subtypes during development. Thanks to intense developmental studies over the last 3 decades (Alaynick, Jessell, and Pfaff 2010), spinal cord development is particularly well described. These data are beginning to be exploited for redirecting adult stem cells into the appropriate cell types.

Investigating the presence and properties of neural precursor cells in humans is a prerequisite for designing regenerative strategies based on the endogenous cellular pool. However, work on adult human tissues is far more complicated than in rodents and raises important ethical, security, reproducibility, organizational and accessibility issues. These issues become exacerbated when research concerning non-pathological, non-fixed and well-preserved CNS is developed. The spinal cord is accessible in organ-donor patients, where thoracic and abdominal organs are removed for transplantation purposes (Dromard et al. 2008). Neurosurgeons could have access to this region of the CNS within hours after the patient is declared brain-dead. This access offers a unique opportunity to characterize the human spinal cord niche and to isolate different neural precursor cell types.

Finally, the spinal cord is an old (oldest ?) component of the CNS but is not just a simple bundle of fibers that connect the brain to the organs and the environment. Recent work has clearly revealed that this primitive CNS region harbors highly-organized internal circuits that are able to display short and long-term plasticity for adapting and memorizing. The spine cord possesses a well-described development and a relatively simple organization with a neural stem cell niche at the center. In addition, its activity can be easily and accurately manipulated by modulating different sensory components (e.g., nociception, temperature,…) and locomotor activity. Therefore, the spinal cord appears as a particularly interesting and simple model for studying the role of adult neural stem cells in CNS physiology. Further characterization of the niche in pathological situations will provide interesting clues on how to utilize this endogenous cell pool to treat spinal cord damage.

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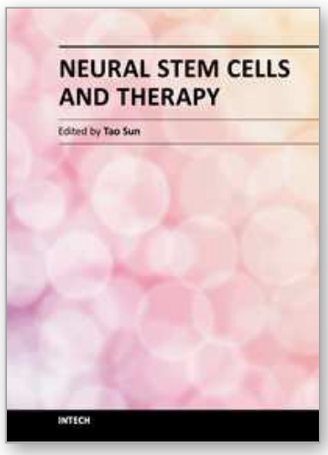

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This book is a collective work of international experts in the neural stem cell field. The book incorporates the characterization of embryonic and adult neural stem cells in both invertebrates and vertebrates. It highlights the history and the most advanced discoveries in neural stem cells, and summarizes the mechanisms of neural stem cell development. In particular, this book provides strategies and discusses the challenges of utilizing neural stem cells for therapy of neurological disorders and brain and spinal cord injuries. It is suitable for general readers, students, doctors and researchers who are interested in understanding the principles of and new discoveries in neural stem cells and therapy.

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